

BROWNING OF SUGAR SOLUTIONS. V. EFFECT OF PH ON
THE BROWNING OF TRIOSES ^{a, b}

(Manuscript received December 28, 1959)

The primary decomposition products of the reducing hexose sugars are generally considered to be glyceraldehyde and its ketoisomer dihydroxyacetone. These two trioses can enolize in alkaline solution to give the 1,2 triose endiol, and it is through this common product that these two compounds are inter-related.

Many investigators (1, 6, 7, 11, 12, 13, 16, 17) have shown that acetol, methylglyoxal and triose-reductone are produced from glyceraldehyde, dihydroxyacetone and from the hexose sugars. Sattler and Zerban (14) have suggested that glyceraldehyde and dihydroxyacetone are intermediates in the formation of acetol methylglyoxal and triose-reductone. Likewise it has been suggested by Zerban and Sattler (18, 19) and by Enders (5) that methylglyoxal, acetol and triose-reductone have a role in the darkening (browning) of hexose and triose sugars. It would appear that the pH of sugar solutions has a pronounced effect on the production of these carbonyls. It has been shown by Meyerhoff and Lohmann (11) and Thornton and Speck (16) that in acid solution methylglyoxal is formed from dihydroxyacetone and by Nodzu *et al* (11) and Prey *et al* (13) that acetol, and by Bauer and Teed (2) and Weygand (17) that triose-reductone are formed in alkaline solutions. These findings together with work done at this laboratory (8) suggest that browning (caramelization) of hexose and triose sugars proceed through the intermediate formation of methylglyoxal, acetol and triose-reductone depending upon the pH of the solution.

This study was undertaken to obtain a better understanding of the intermediates formed in the browning of the trioses, dihydroxyacetone and glyceraldehyde, in solutions of pH 4 to 11.

EXPERIMENTAL

Materials. *Triose reductone:* Triose reductone was obtained from Wallerstein Laboratories as a dark brown amorphorous material which was purified by sublimation under vacuum (2 mm) at 40° C. The white sublimate was stored under nitrogen.

Acetol: Acetol was obtained from Bios Laboratory and purified by vacuum distillation. The fraction boiling at 45° C (10 mm pressure) was collected and stored over Drierite.

Methylglyoxal: Methylglyoxal was obtained from Bios Laboratory as a 30% aqueous solution. This was extracted with 2 portions of diethyl ether. The ether extracts were combined and the ether evaporated at reduced pressure. The resulting liquid distilled at 50° C (15 mm). The neutral straw-colored distillate was dissolved in 3 volumes of water and stored at 4° C.

^a Presented at the 136th National Meeting of the American Chemical Society, Atlantic City, New Jersey, September 13-18, 1959.

^b Based on an investigation reported in a thesis submitted by H. G. Lento to the Graduate School of Georgetown University as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

^c Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, Philadelphia 18, Pennsylvania.

Glyceraldehyde: This was obtained from Nutritional Biochemicals.

Dihydroxyacetone: This was obtained from Nutritional Biochemicals.

The purity of the triose-reductone sublimate was 99.4% determined titrimetrically with 2,6-dichlorophenolindolphenol (9).

The concentration of the acetol and methylglyoxal solutions and the purity of the glyceraldehyde and the dihydroxyacetone were determined by periodic oxidation (4) and as the 2,4-dinitrophenylosazones. The concentration of the acetol and the methylglyoxal solutions were 85.2% and 35.5% respectively. The purity of the glyceraldehyde and that of the dihydroxyacetone was 99.3% and 99.5% pure, respectively.

Two-tenths molar aqueous solutions of each of the 5 carbonyls were prepared and constituted the stock solutions.

Buffers: The phosphate buffer solutions were 0.25 molar disodium acid phosphates adjusted to the desired pH with either one normal sodium hydroxide or with 85% phosphoric acid.

Procedure. Due to the reactivity of the carbonyl compounds with oxygen, care was taken to displace the air completely from all containers with nitrogen. The water used was first boiled to degas it and then swept with nitrogen. All solutions were stored under nitrogen.

The browning reactions were carried out in the reaction vessel and sampler shown in Figure 1.

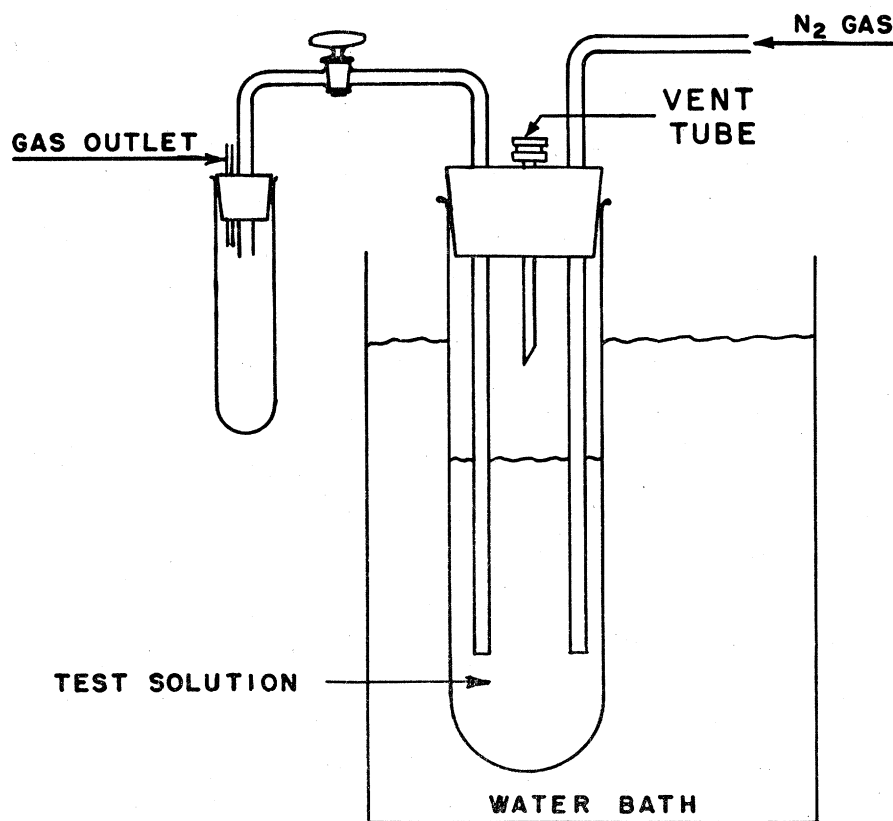


Figure 1. Sampling apparatus.

BROWNING OF SUGAR SOLUTIONS. V.

With the rubber stopper removed, 90 ml of the appropriate buffer solution was placed in the large tube (reaction vessel) and the tube lowered into a 100° C water bath to a depth to cover the buffer in the tube. The stopper was replaced and nitrogen swept through the system to free it from oxygen. Ten ml of the 0.2M stock solution of the particular carbonyl was then added to the hot buffer solution by partially removing the stopper and inserting the tip of the pipet. The stopper was tightly seated and samples were withdrawn at different times by closing the vent tube with a finger tip thus causing the nitrogen gas to force the carbonyl buffered solution, approximately 10 ml, over into the sample tube which was kept chilled in an ice water bath. The sample tube was then disconnected from the system, stoppered and the spectral transmission of the carbonyl-buffer solution measured at 450 mμ using a Coleman Spectrophotometer.

RESULTS AND DISCUSSION

The rate at which color was produced at a given pH by each of the carbonyls was determined by measuring the absorbance of the carbonyl-buffered solutions heated at 100° C for different lengths of time. Since the rate of color formation in the acid solutions was slower, samples were heated for longer periods of time. Since all solutions were adjusted to contain 0.02M of the respective carbonyl the rates and amounts of color produced were characterized by plotting the absorbances of each of the solutions versus heating times to obtain the curves shown in Figures 2 and 3.

Triose reductone has been implicated in the alkaline degradation of reducing sugars and their caramelization reaction products (18, 19). These studies have shown that heated, deoxygenated solutions of reductone produced no color over the pH range of 4 to 11. This is concordant with observations regarding other reductones in which the dehydroforms were the ones which browned (3).

The greatest amount of color was produced by the other 4 carbonyls at the highest pH with solutions of methylglyoxal browning fastest and dihydroxyacetone the least.

The slowest rate of browning for the 4 carbonyls was in the acid range. At pH 4 and 5 the amounts of color produced by glyceraldehyde and dihydroxyacetone were so small (0.02 absorbance even after 75 min at 100° C) that rates of their browning were not plotted. However, when these solutions were heated for much longer periods of time (2 hr) it was shown that the rate of color production was linear.

Since all of the curves, Figures 2 and 3, are linear their slopes were taken as a measure of reaction rates. From these were obtained reaction constants. These reaction constants were then plotted as a function of pH to give the curves in Figure 4.

The slope of these curves, rate constants of color production versus pH, show that the curve for acetol is steepest, indicating the most rapid change in color with pH increase. The curve for methylglyoxal has the least slope, indicating the lowest increase in reactivity with increasing pH. The curves for dihydroxyacetone and glyceraldehyde are identical and superimposed. The slope of these curves indicates that the rate constants (color formation) is not a straight line function. It is significant that a change in the dihydroxyacetone-glyceraldehyde curves occurs in the pH region of 8-9. Above pH 9 and below pH 8 the slopes of the curve are quite different, indicating that changes are occurring in both the dihydroxyacetone and glyceraldehyde solutions as the pH of the solutions is changed.

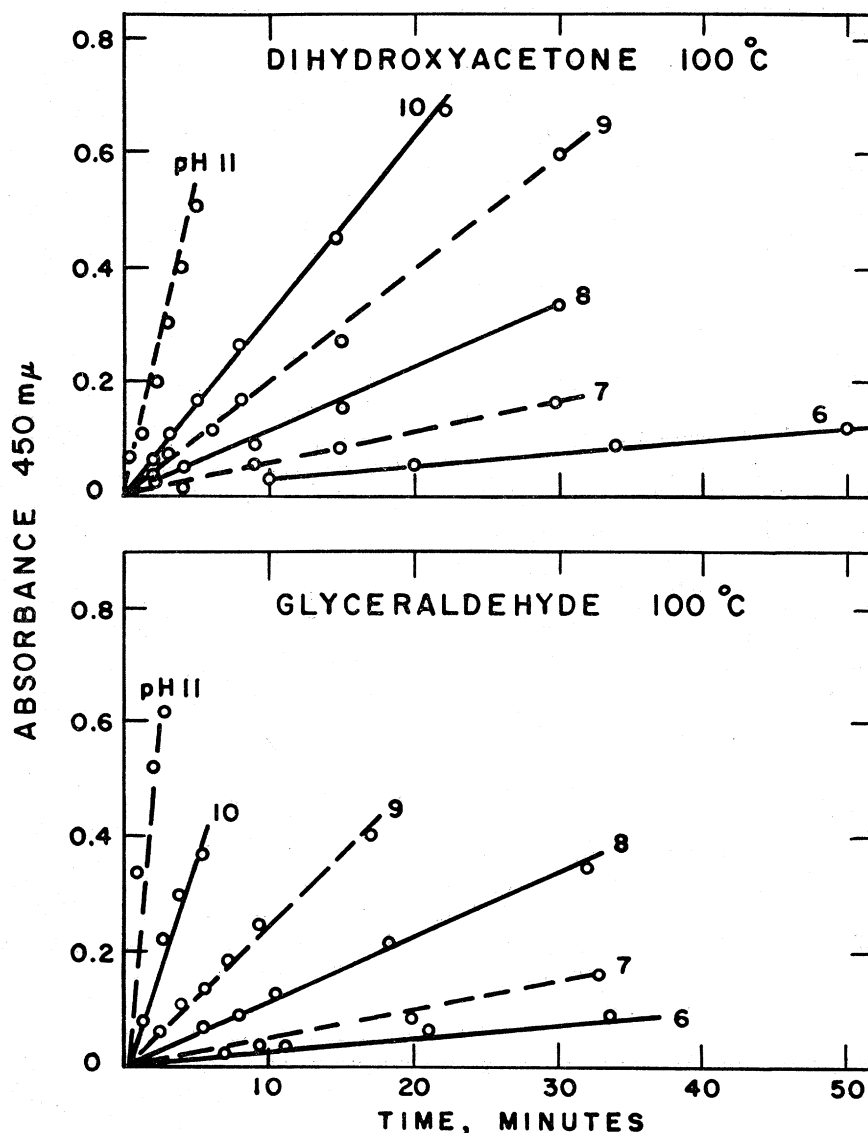


Figure 2. Rates of coloration of dihydroxyacetone and glyceraldehyde at various pH values.

That portion of the curve between pH 4 and pH 8 is similar to, but less steep than, that for methylglyoxal except for that portion close to pH 4. This strongly suggests that the formation of color is due, at least in part, to the formation of some constituent from both dihydroxyacetone and from glyceraldehyde. This formed constituent is no doubt methylglyoxal since it has been shown by earlier work in this laboratory (8) that the formation of methylglyoxal from these two triose sugars is increasingly favored as the pH is decreased to 4. Previous work here (8) has also shown that as the pH of

BROWNING OF SUGAR SOLUTIONS. V.

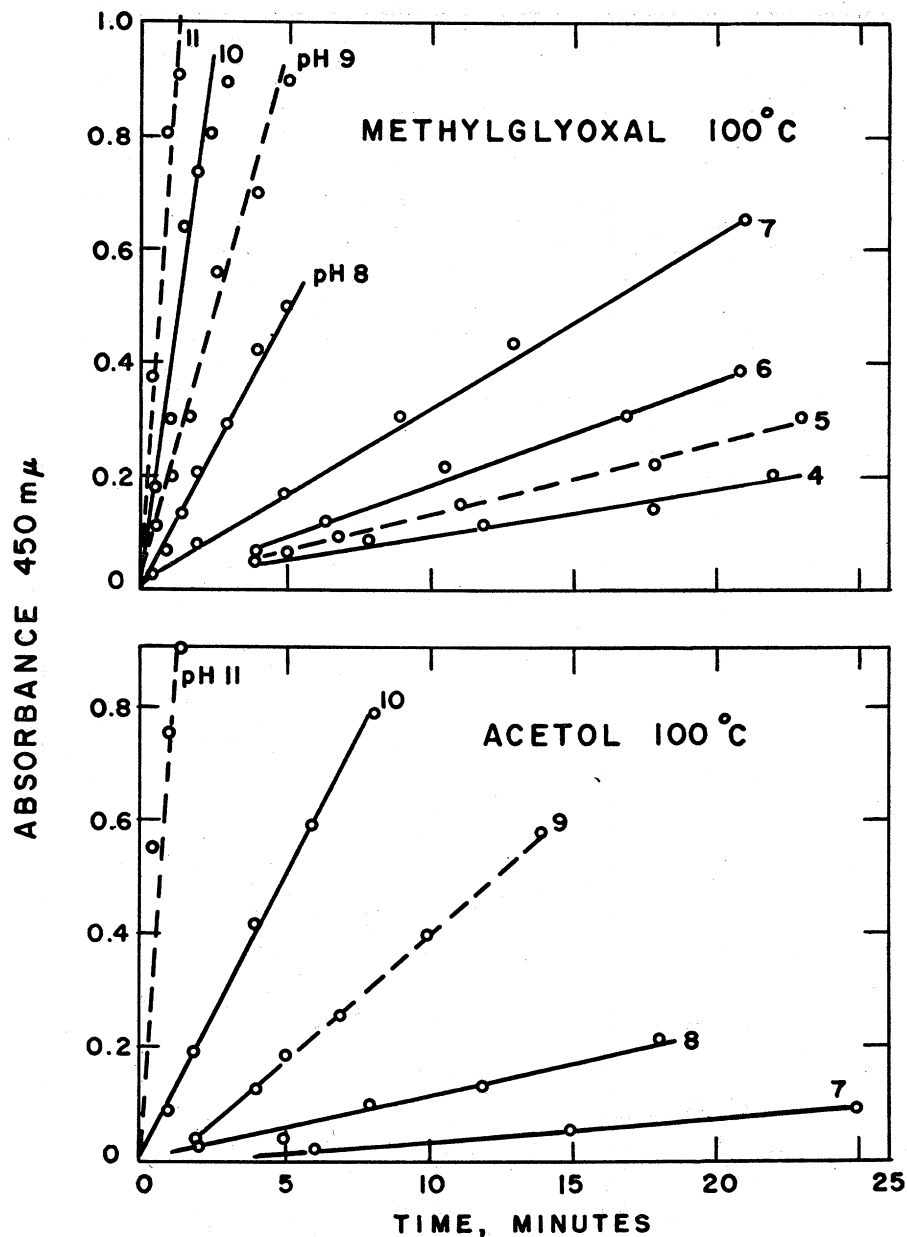


Figure 3. Rates of coloration of methylglyoxal and acetol at various pH values.

solutions of these two sugars is decreased below 7 little or no acetol is formed. This indicates that methylglyoxal is being formed and that it is involved in the acid browning of dihydroxyacetone and of glyceraldehyde.

Above the inflection point of the curve, pH 8-9, the change in the coefficient rate of browning with pH again suggests the formation of a constituent from the two triose sugars, which is not methylglyoxal but very probably acetol.

Previous work has shown (8) that as solutions of these two sugars become more alkaline the formation of acetol is favored. With conditions favorable for the production of acetol coupled with the fact that alkaline solutions of acetol brown rapidly, the activation (browning) curve becomes steeper as pH 11 is approached.

It is, therefore, assumed that the successive reactions which bring about color formation in heated solutions of dihydroxyacetone and glyceraldehyde is due to the formation of methylglyoxal and/or acetol whose production is dependent upon the pH of the solution.

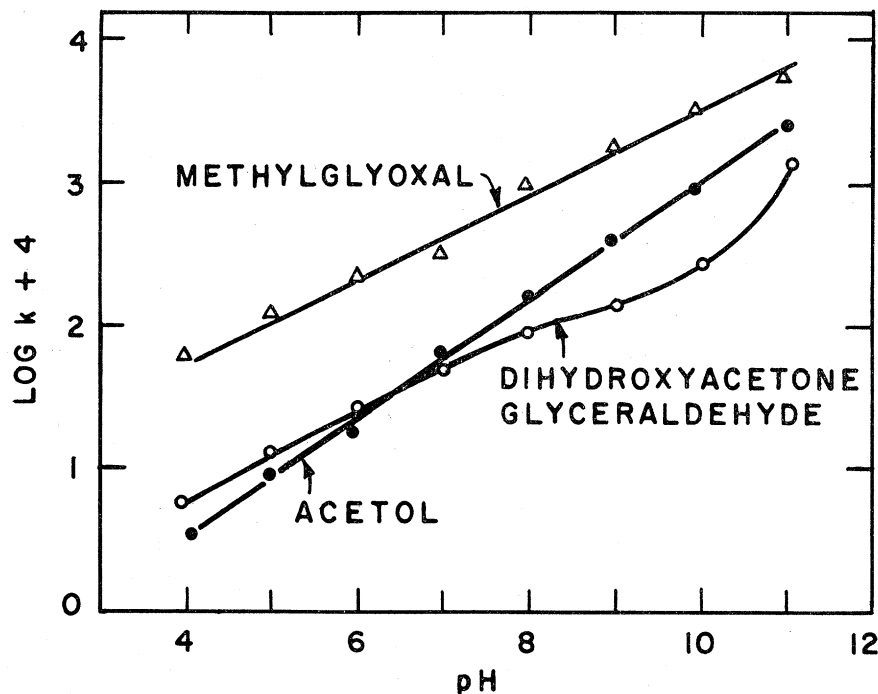


Figure 4. Relationship between pH and log k for rate of coloration.

LITERATURE CITED

1. BAUDISCH, O., AND DEUEL, H. J. A new test for carbohydrates. *J. Am. Chem. Soc.*, **44**, 1585 (1922).
2. BAUER, H. F., AND TEED, C. Degradation of D-glucose-1-C¹⁴ to triose-reductone. *Can. J. Chem.*, **33**, 1824 (1955).
3. DULKIN, S. I., AND FRIEDMAN, T. E. Dehydroascorbic acid dehydroreductic acid browning reactions. *Food Research*, **21**, 519 (1956).
4. DYER, J. Use of periodate oxidation in biochemical analysis. *Methods of Biochemical Analyses*, **3**, 111 (1956).
5. ENDERS, C., AND MARQUARDT, R. Methylglyoxal als Zwischenprodukt der Melanoidin und Karamelbildung. *Naturwissenschaften*, **29**, 46 (1941).
6. VON EULER, H., AND HASSELQUIST, H. Reductone—Ihre chemische Eigenschaften und biochemische Wirkungen. 1950. Stuttgart.
7. EVANS, W. L., AND O'DONNELL, D. The mechanism of carbohydrate oxidation. X. The action of potassium hydroxide on mannose. *J. Am. Chem. Soc.*, **50**, 25 (1928).

BROWNING OF SUGAR SOLUTIONS. V.

8. LENTO, H. G., UNDERWOOD, J. C., AND WILLITS, C. O. Browning of sugar solutions. IV. The effect of pH on the steam volatile decomposition products of reducing sugars. *Food Research*, **25**, 750 (1960).
9. LORENZ, A., AND ARNOLD, C. Standardization of 2,6-dichlorophenolindophenol with ferrous compounds. *Ind. Eng. Chem., Anal. Ed.*, **10**, 687 (1938).
10. MEYERHOFF, O., AND LOHMANN, K. Über die enzymatische Gleichgewichtsreaktion zwischen hexosediphosphorsäure und Dioxyacetonephosphorsäure. *Biochem. Z.*, **271**, 89 (1932).
11. NODZU, R., MATSUI, K., GOTO, R., AND KUNESHIK, S. On the action of phosphate upon hexoses. III. Simultaneous formation of acetol and pyruvic acid from glucose. *Min. Coll. Soc. Kyoto Imp. Univ.* **20A**, 197 (1937).
12. NODZU, R., AND GOTO, R. The action of weak alkali on glucose. *Bull. Chem. Soc., Japan*, **15**, 209 (1940).
13. PREY, V., WALDMAN, E., BERBALLE, F., AND LUDWIG, F. Zur Kenntnis des glycerin-aldehyde. *Monatsh. Chem.*, **84**, 551 (1953).
14. SATTLE, L., AND ZERBAN, F. W. Glucic acid and the breakdown of sugars. *Sugar*, **42**, (12) (1947).
15. THORNTON, B., AND SPECK, J. C. Fluorometric determination of pyruvaldehyde. *Anal. Chem.*, **22**, 899 (1950).
16. WERLE, E., AND STIESS, P. Quantitative Bestimmung des Acetols mit besonder Berücksichtigung seiner Bildung beim Alkalischen Zuckerabbau. *Biochem. Z.*, **321**, 485 (1951).
17. WEYGAND, F. Papierchromatographische Untersuchung der Reductonbildung. *Arkiv. Kemi*, **3**, 11 (1950).
18. ZERBAN, F. W. The Color Problem in Sucrose Manufacture. Technological Report No. 2, Sugar Research Foundation, Inc. (1947).
19. ZERBAN, F. W., AND SATTLE, L. Unfermentable reducing substances in molasses. *Intern. Sugar J.*, **57**, 71 (1955).